IN THE CLAIMS

1. (Amended) A method of using Raman imaging microscopy for evaluating drug action within living cells comprising the steps of:

first measuring a Raman spectrum of said drug to determine a pretreatment fingerprint of said drug;

second measuring a first direct Raman image of said living cells at a predetermined wavelength on said Raman spectrum in order to determine a pretreatment background of said living cells;

treating a culture of said living cells with said drug to obtain treated living cells;

next measuring a second direct Raman image of said treated living cells at said predetermined wavelength on said Raman spectrum to obtain post-treatment images of said living cells;

processing said post-treatment images and pretreatment backgrounds to obtain processed post-treatment images and processed pretreatment backgrounds to evaluate said drug action within said living cells; and

dividing said processed post-treatment images by said processed pretreatment backgrounds according to the formula $s'(x,y)/ s(x,y) = K'(x,y)/ K(x,y)$, where $s'(x,y)$ is said processed post-treatment image, $s(x,y)$ is said processed pretreatment background, $K'(x,y)$ is the Raman scattering coefficient for said processed post-treatment image, and $K(x,y)$ is the Raman scattering coefficient for said processed pretreatment background, wherein said dividing step is used to obtain a ratio of images which indicate the changes of said living

cells after said treating whereby said changes are used to determine said drug action.

2. (Original) The method of using Raman imaging microscopy for evaluating drug action within living cells of Claim 1 wherein said ratio of images is obtained for various times to determine different depths within said living cells.

3. (Original) The method of using Raman imaging microscopy for evaluating drug action within living cells of Claim 2 further comprising the step of stacking said ratio of images to obtain a three dimensional Raman image.

4. (Original) The method of using Raman imaging microscopy for evaluating drug action within living cells of Claim 3 wherein said processing step comprises determining the Raman scattering coefficient of an imaging area from a recorded image for said post-treatment images and said pretreatment fingerprints.

5. (Canceled)

6. (Amended) The method of using Raman imaging microscopy for evaluating drug action within living cells of Claim 4 wherein said processing step further comprises determining said Raman scattering coefficient by compensating for any point spread function.

7. (Canceled)

8. (Original) The method of using Raman imaging microscopy for evaluating drug action within living cells of Claim 2 further comprising the step of plating said living cells on a dish coated with Raman inactive material to prevent Raman signals coming from said dish during said measuring steps.

9. (Amended) The method of using Raman imaging microscopy for evaluating drug action within living cells of Claim 8 wherein said treating step occurs for a specific period of time.

10. (Original) The method of using Raman imaging microscopy for evaluating drug action within living cells of Claim 9 wherein said measuring steps utilize a system having a Raman microscope with a 30 mw diode laser at 780 nm as the excitation source.

11. (Original) The method of using Raman imaging microscopy for evaluating drug action within living cells of Claim 10 wherein said system is stabilized on a vibration controlled table.

12. (Original) The method of using Raman imaging microscopy for evaluating drug action within living cells of Claim 11 wherein said living cells are obtained with a water immersion, high infrared transmission objective.

13. (Original) The method of using Raman imaging microscopy for evaluating drug action within living cells of Claim 12 wherein said transmission objective has a transmission coefficient of the lens at 780 nm excitation wavelength of 71%.

14. (Original) The method of using Raman imaging microscopy for evaluating drug action within living cells of Claim 13 wherein said lens has a numerical aperture of 0.90.

15. (Amended) A method of using Raman imaging microscopy for evaluating drug action within living cells comprising the steps of:

first measuring a Raman spectrum of said drug to determine a fingerprint of said drug;

second measuring a first direct Raman image of said living cells at a preselected Raman peak of said drug to determine a pretreatment background of said living cells and recording a corresponding visible image of said living cells;

treating a culture of said living cells with said drug to obtain treated living cells;

next measuring a second direct Raman image of said treated living cells at said preselected Raman peak of said drug to obtain post-treatment images of said living cells and recording a corresponding visible image of said living cells;

processing said post-treatment images and said pretreatment background to obtain processed post-treatment images and processed pretreatment backgrounds, respectively, to reduce imaging artifacts;

comparing said processed post-treatment images with said processed pretreatment backgrounds to resolve the changes due to drug distribution within said living cells after said treatment and

determining said drug distribution relative to cellular organelles of said living cells by comparing said Raman images with said corresponding visible images, whereby said drug distribution is used to evaluate said drug actions within said living cells.

16. (Original) The method of using Raman imaging microscopy for evaluating drug action within living cells of Claim 15 wherein said processing step further comprises one or more of the following:

a) smoothing noise;

b) reducing blurring by the optical system;

c) correcting non-uniform illumination effects; and/or

d) eliminating fluorescence background from said post-treatment images.

17. (Original) The method of using Raman imaging microscopy for evaluating drug action within living cells of Claim 15 wherein using said drug distribution to evaluate said drug action within said living cells comprises one or more of the following process steps:

a) estimating the cellular uptake of said drug;

b) detecting said drug distribution within said living cell;

c) determining the local binding and biochemical pathway of said drug;

d) determining the cellular resistance of said living cells to said drug;

e) analyzing the pharmacokinetics of said drug; and

f) determining the metabolism of said drug.

18. (Original) The method of using Raman imaging microscopy for evaluating drug action within living cells of Claim 15 further comprising the step of plating said living cells on a dish coated with Raman inactive material to prevent Raman signals coming from said dish during said measuring steps.

19. (Original) The method of using Raman imaging microscopy for evaluating drug action within living cells of Claim 15 wherein said treating step incorporates the use of a drug delivery system.

20. (Original) The method of using Raman imaging microscopy for evaluating drug action within living cells of Claim 1 wherein said evaluation of said drug action within said living cells comprises one or more of the following process steps:

a) estimating the cellular uptake of said drug;

b) detecting said drug distribution within said living cell;

c) determining the local binding and biochemical pathway of said drug;

d) determining the cellular resistance of said living cells to said drug;

e) analyzing the pharmacokinetics of said drug; and

f) determining the metabolism of said drug.

21. (Original) The method of using Raman imaging microscopy for evaluating drug action within living cells of Claim 1 wherein said ratio of images is used to quantify the local concentration of said drug within said living cells.